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TITLE: Services to Operate a Hemoglobin Production Facility and a Red Blood Cell Storage Laboratory

PRINCIPAL INVESTIGATOR: Lloyd E. Lippert

CONTRACTING ORGANIZATION: The Bionetics Corporation
Hampton, Virginia 23669

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13. ABSTRACT *(Maximum 200 words)*

A research laboratory to support red blood cell preservation research, a facility to manufacture acellular hemoglobin solution and a quality control laboratory to support the hemoglobin production were established at the Blood Research Detachment, 1413 Research Boulevard, Rockville, MD with equipment from the Letterman Army Institute of Research. Procedures were established, equipment maintained and staff trained to support both *in vitro* and *in vivo* evaluation of preservative solutions which will extend the shelf life of liquid stored red blood cells. Likewise, standard operating procedures were established, equipment was maintained, staff were trained, and 180.5 kg of hemoglobin solution containing 16.2 kg of hemoglobin which met or exceeded contract specifications was manufactured in the hemoglobin production facility (HPF). Three lots of material were manufactured for the US Navy lipisome encapsulated hemoglobin program. Ten hemoglobin production process improvements were implemented and a series of process validation studies were initiated. Substantial progress has been achieved in bringing the HPF into closer compliance with current Good Manufacturing Practices, a necessary step of Phase I and II clinical trials. Systems are in place to support the Blood Research Detachment's combat casualty care research mission.

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L E Lippert 16 OCTOBER 1995

PI - Signature Date

Lloyd E. Lippert, PhD, SBB(ASCP)

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ANNUAL REPORT - DAMD17-94-C-4154

INTRODUCTION

Nature of the Problem:

Because combat is synonymous with bloodshed and blood replacement saves lives, The US Army Medical Research and Material Command maintains facilities and programs to develop improved blood products and blood substitutes. The US Army has for decades conducted research in red blood cell preservation and the production of acellular hemoglobin solutions for use in combat casualty care. From 1974 through 1992, that research took place at the Letterman Army Institute of Research (LAIR) located at the Presidio. The LAIR facility was closed as the result of Base Realignment and Closure actions and the Blood Research Detachment was relocated to leased laboratory space at 1413 Research Blvd., Rockville, Maryland. On 19 September 1994 The Bionetics Corporation (TBC) was awarded a contract to operate and maintain equipment and provide technical support to the Blood Research Detachment. For the purposes of this report, contract activities will be divided into 3 categories. The first is a Hemoglobin Production Facility (HPF) which consists largely of installed, custom made equipment, most of which is located in a class 10,000 clean room. TBC assembled a team of 4 full-time and 1 part time employees to operate and maintain all equipment associated with those activities. Integral to the operation of the HPF is an analytical chemistry laboratory which provides the quality control and characterization testing of the hemoglobin solutions. This activity will be referred to as Quality Control (QC) within this report and is supported by 1 full-time and 1 part time employee. The third category of activity supported is blood cell preservation research. This activity is encompassed within the Blood Storage Laboratory (BSL), and a fully equipped red cell research laboratory staffed by 3 full-time employees. The Blood Banking Specialist member of this team is also the Project Manager.

The Background of the Previous Work:

HPF: The production of an acellular hemoglobin solution, around which the HPF was designed, is based on biochemical modification of stroma-free hemoglobin as described in the literature.[1,2,3,4] The modified hemoglobin, or the unmodified intermediate, is purified and suspended in a crystalloid solution, dispensed into plastic bags and frozen at -80°C.

BSL: The maximum shelf life of red blood cells stored at refrigerator temperatures using the currently Food and Drug Administration (FDA) licensed anticoagulant, preservative solutions is 42 days after collection. Work by Merriman *et. al.*[5,6] and Greenwalt, Dumaswala and colleagues[7,8] indicates potential for extended storage. It is estimated that 1 million units of blood expire per year in the United States and one-third would be used if expiration were extended to 8 weeks.[9] Extended shelf-life of liquid stored blood would have significant utility to the Armed Services Blood Program as it supports the Department of Defense blood transfusion requirements world-wide.

The Purpose of the Present Work:

HPF: The purpose of the present work is to produce a large quantity (150 liters) of a highly purified, well characterized acellular hemoglobin solution. The material, in turn, is used by the government to support further research to define the mechanisms of toxicity of blood substitutes and complete evaluation for potential Phase I and Phase II clinical trials and eventual commercial development. A second purpose is to optimize the existing process.

QC: The Quality Control laboratory supports the testing needs of the HPF.

BSL: The Blood Storage Laboratory evaluates the effectiveness of novel candidate red blood cell anticoagulant preservative solutions and their potential for further development.

METHODS

HPF: The overall method was to evaluate current operations and apply the combined team experience and training to bringing operations into close or full compliance with current Good Manufacturing Practices (cGMP).[10] Compliance with cGMP's is necessary for Phase I & II developmental therapeutic drugs and devices and is the basis for providing assurance that the system will consistently produce a hemoglobin product meeting the determined specifications and quality desired. Activities focused on 3 areas: documentation, equipment repair and maintenance and process improvement.

Documentation:

A. In accordance with cGMP's, a system for tracking and documenting all protocols, hereafter referred to as Standard Operating Procedures (SOP's), was established. These SOP's are written with such detail that all appropriately trained individuals can read a specific protocol and be able to perform that task consistently. The SOP's addressed administrative and well as technical procedures.

B. A Blood Inventory Control system was established to track all outdated red blood cell units received for manufacture of hemoglobin solution. This system begins with each individual unit physically inspected and the unit number compared to the packing slip. Once this is completed, the units are entered into a database which tracks the ABO group, the shipping site, expiration date, and the unit's disposition by the HPF. The timely retrieval of disposition is imperative should a donation be identified for HIV look-back. The database is also the source for any required reporting.

C. A Raw Material Control system has been established for all critical supplies and chemicals which come directly in contact with the product. This system maintains a comprehensive inventory of all items necessary for the hemoglobin production. This system also ensures that the same item from the same vendor is used each time a hemoglobin lot is produced. The documentation also includes certificates of analyses for each raw material.

D. A Hemoglobin Inventory Control system was established in order to account for any inventory which was transported from LAIR and for the inventory generated at this facility. This was accomplished by setting up specific areas in the freezers for each lot, documenting lot specific inventory location and quantities, documenting on the outside of each freezer the inventory within the freezer, and locking all freezers for limited access. An overall hemoglobin inventory log is generated and updated as necessary.

Equipment Repair and Maintenance :

A. Because the HPF equipment had been recently installed and received little or no operational testing at the current site, all systems were thoroughly evaluated for immediate repairs and longer term maintenance. Repairs, both large and small, were initiated. The most significant was the bioreactor which required near total replacement of seals and gaskets plus replacement of bent mixer shaft.

B. A preventive maintenance program was established. Where possible, contract staff were utilized. Some equipment required vendor or original equipment manufacturer (OEM) maintenance. An array of coverage was applied ranging from simple calibration or preventive maintenance to full-service maintenance including parts and labor. The vendors also provided the calibration and maintenance documentation required for compliance with cGMP's. The type of agreement chosen was determined by balancing the criticality of the item, the likelihood of failure, the existence of alternatives with the costs.

Process Improvement/Optimization: Opportunities for process improvement and optimization were identified by performing the manufacturing process as provided by the government and comparing to other procedures and techniques commonly used by industry. All procedures from cleaning the surfaces and equipment to filling the final container were examined. Ten significant process changes were incorporated during this period; details of the process changes are included in the Results section.

QC: In the Quality Control/Analytical Chemistry Laboratory, assays were modified to fit the needs of the HPF and to conform with GMP's and industry standards of practice.[10,11] Methods were researched and procedures documented as Standard Operating Procedures. Appropriate controls were incorporated within the protocols for in-house assays lending a high degree of confidence to the assay. Performance of the assays in the expected range was verified with available standards. Consequently, tighter control over the final product prepared in the HPF was established. Some assays were identified for performance at a reference laboratory because the necessary equipment was unavailable or it was more cost effective to utilize an outside laboratory. Environmental and personnel monitoring has been instituted during the manufacturing and filling processes to check the effectiveness of cleaning procedures, gowning procedures, room air filtration and other measures designed to maintain sterility. Validation studies were initiated in the HPF to ensure the safety and consistency of the hemoglobin product. These studies included testing the filling operation for sterility, the effectiveness of cleaning with all agents utilized in the HPF, for residual detergents used in equipment cleaning, and verifying the steam pressures and time used in tanks and vessels cleaning. These validations will assure that processes which have been implemented are performing as expected.

BSL: The laboratory utilizes an array of pertinent *in vitro* and *in vivo* tests. The *in vitro assay* developed are those classically used in the evaluation of the red cell storage lesion. The laboratory is also capable of performing *in vivo* red cell survival studies. The measure of efficacy specified by the FDA is the 24 hour survival of stored red blood cells. This is measured by recovering ^{51}Cr labeled stored red blood cells from a circulatory volume precisely determined

using ^{99m}Tc labeled, fresh red blood cells. The mean survival of the stored cells must be at least 75%.

RESULTS

General and Administrative: A copy of the Table of Contents for the SOP's which have been written thus far for all contract operations is attached as Appendix 1. Thirty nine SOP's are completed; an additional 30 are in some intermediate stage of preparation.

HPF: A total of 180.5 liters of hemoglobin solution containing 16.2 kg hemoglobin was produced which met or exceeded the specifications. Appendix 2 is an account of all hemoglobin manufactured at the WRAIR facility under the jurisdiction of Bionetics Contract C411. The three lots of stroma-free and seven lots of cross-linked hemoglobin were all tested for nine and 12 analytes respectively. A summary of results for each analyte is as follows:

Assay	Units	Stroma Free Hb	Cross Linked Hb
Total Hemoglobin	g/dl	13.7-15.8	6.78-14.96
Met Hemoglobin	%	0.58-0.64	1.40-4.74
P_{50}	Torr	5.25-11.25	21.5-29.5
FPLC	%	NA	>80-<100
HPLC	%	NA	>>95
pH	NA	7.25-7.42	6.90-7.86
Osmolarity	mOsm	49-263	
Free Iron	mol Fe/mol Heme	$1.22\text{-}4.39\times10^{-5}$	$1.67\text{-}3.55\times10^{-5}$
Phospholipid	µg/ml	<0.1-0.54	0.125-1.0
LAL	Eu/ml	<0.03-0.25	0.125-0.25
Sterility	NA	PASS	PASS
Pyrogen	NA	NA	PASS

Abbreviations:

FPLC = fast protein liquid chromatography

HPLC = high performance liquid chromatography

LAL = *limulus* amebocyte lysate

Eu = Endotoxin Units

The Certificates of Analysis for each lot of material produced under contract, data summarized above, are included in this report at Appendices 3 through 11. A copy of the most current inventory has been attached as Appendix 12; this inventory includes materials other than those produced under contract. During the course of the 1995 contract year, 755 units of expired packed red blood cells were used in the preparation of hemoglobin; an additional 601 units were received and either destroyed or used for other research by the BRD. A copy of the Blood Source Report is attached as Appendix 13.

A number of process changes have been incorporated into the HPF which have resulted in both time and cost savings from that initially transferred from the LAIR facility. These changes have been outlined in table format below. The amount of time saved during the production based on these process changes is Day 1: 5 hours, Day 2: 2.5 hours, and Day 3: 6 hours from a total production time of approximately 45 to 32 hours. The overall material cost savings are negligible since the overall changes balance. However, efficiency was greatly improved because total manhours required per manufacturing run has been substantially reduced and the quality of the product is higher.

HEMOGLOBIN PRODUCTION PROCESS CHANGES WHICH INCREASED EFFICIENCY AND OPTIMIZED THE PROCEDURE

Area of Process Change	Process Change	Overall Change
RBC Pooling	Pooling units the morning of production → Pooling units the day before production	3 hours saved on the first day of production.
Cleaning Supplies	Germicidal → Germicidal, Fungicidal, Virucidal, Bacteriocidal, Tuberculocidal	Safety of Hb product.
Deoxygenation Step	1 membrane → 2 membranes Incorporated a fiber optic system.	2 hours saved on the second day of production.
3μ Filtration	1x3μ filter → 3x3μ filters and 1 pre-filter	3 hours saved on the third day of production.
RBC Wash	20X Wash → 3X Wash	1½ hours saved on the first day of production. Cost savings.
HEPES Buffer	HEPES → 5N NaOH	Replace with a USP item. Cost Savings.
DBBF	Deoxygenation → No Deoxygenation	1/2 hour saved on the second day of production.
Phosphate Buffer (Oxygenation Buffer)	Buffer → 1 Oxygenator Membrane	3 hours saved on the third day of production.
Ringer's Acetate w/ Calcium	Ringer's Buffer → Saline	Cost Savings.
Final Product Bag	Baxter → Stericon Vendor	Ease of handling during filling process. Esthetics.

QC: The QC laboratory has established the following assays to characterize the final product:

pH

hemoglobin concentration (both total and met-hemoglobin)

osmolarity

cross-linking efficiency by HPLC

P_{50}

quantitation of endotoxin by the *limulus* amebocyte lysate (LAL) assay

sterility testing

Reference laboratories are used to evaluate cross-linking efficiency by FPLC, test for residual free iron and phospholipid and perform the rabbit pyrogen testing. The endotoxin and microbiological (sterility) tests have been validated by outside GMP licensed laboratories. This was conducted to ensure the in-house assays were operating properly and to assess the bacteriostatic and fungistatic properties of the hemoglobin product which would create false positive or false negative results.

The QC laboratory also provided operational support to the HPF before, during and after production. The in-process assays for pH, total hemoglobin concentration and endotoxin levels needed to verify function or adjust the process were provided during production. Before and after production runs the QC laboratory supported the HPF by testing samples taken after cleaning for endotoxin, microbial growth and residual cleaning agent prior to reuse. Monthly testing of the HPF purified water supply has been instituted as an in-house assay; the water testing is in accordance with the USP[12] and is substantially less expensive than the same testing provided by reference laboratories. Testing of all buffers used in the HPF prior to their use in production has been instituted. Environmental and personnel monitoring has been instituted during the manufacturing and filling processes.

BSL: The contract personnel developed and validated all procedures required to support

the initial protocols. This was accomplished after sorting, organizing and ordering supplies and reassembling equipment components, reestablishing equipment function and establishing maintenance procedures for equipment which had been in storage for approximately a year, moved to a renovated laboratory and unused until the arrival of the contract staff. The contract staff also received training in use of radioisotopes and performance of red cell survival studies. However, no data was generated during this period because the first protocol to utilize red cell survival studies received final approval during the last week if the fiscal year. The first set of volunteers will be screened during the third week of October 1995.

DISCUSSION

HPF: The HPF is fully operational and staff is trained and experienced. The hemoglobin solution produced exceeds the amount specified in the contract and meets or exceeds the required specifications of quality and purity. Furthermore, the staff was also able to respond to a new requirement, of providing hemoglobin to the US Navy Lipisome Encapsulated Hemoglobin program, and produce material tailored to their specifications. The systems and procedures are in place to continue producing hemoglobin solutions of consistent high quality and respond to new requirements.

QC: The Quality Control laboratory provided the support necessary for in-process operation of the HPF and provided the assays required to characterize the final product. The continuing validation efforts will provide the foundation for assurance of a high quality material suitable for Phase I and Phase II clinical trails. Systems are in place to develop additional assays such as quantitation of residual diasprin cross-linking reagent in the final product and to reevaluate the assays, currently performed by reference laboratories, for in-house development and performance.

BSL: The Blood Storage Laboratory is poised to evaluate candidate anticoagulant preservative solutions which will extend the shelf life of liquid stored blood and generate the

fundamental data required for FDA licensure. The BSL will play a critical role in this research effort because they are one of only a limited number of centers which can provide the *in vivo* red cell survival data required by the FDA. This laboratory will also be a resource to support both the Armed Service Blood Program operational needs and the more basic research relevant to red cell membrane structure and function, red cell senescence and related issues.

CONCLUSION

The contract staff has supported the BRD by operating and maintaining the HPF and producing well characterized, high quality hemoglobin solutions in quantities which met the contract requirements. Staff are trained and the necessary quality control and documentation systems are in place to support both existing and new requirements. Substantial progress has been made toward cGMP compliance. The BSL staff has likewise established the required assays and procedures to support blood preservation research. Staff are trained and systems are in place to support specific protocols when all regulatory approvals are completed. The BRD mission has been supported.

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THE BIONETICS CORPORATION
Standard Operating Procedures
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700- RADIOSAFETY

SOP. TBL [Pilot Plant Disk]
Revision Date 9/29/95

HEMOGLOBIN PRODUCTION FACILITY
FY 1995 CONTRACT YIELDS
BIONETICS

Lot No.	Final Concentration (g/dl)	Actual Weight[1] (kg)	Final Weight[2] (kg)	Total Grams Hemoglobin
950111	13.7	27	27	3700
950207	6.78	23.2	23.2	1573
950314	8.24	29.7	29.7	2447
950404	7.48	24.6	24.6	1840
950516-01	14.0	4.4	7	616
950516-02	12.3	6.3	11.6	775
950725	14.96	13.64	25.4	2041
950808	15.8	20.3	32	3207
Total		149.14	180.5	16.199 kg

[1] Actual weight manufactured.
[2] Equivalent weight of 8 g/dl contract specification.

2 October 1995

ORIGINAL

COPY

CERTIFICATE OF ANALYSIS
FINAL CONTAINER
CROSS LINKED HEMOGLOBIN

LOT #: J94319

ASSAY	RESULT
Total Hemoglobin	8.63 g/dl
Met Hemoglobin	2.72 %
P_{50}	22.6 Torr
FPLC	ND
HPLC	69.2 %
pH	7.81
Osmolarity	292 mOsm
LAL	1.0 EU/ml
Sterility	ND
Pyrogen	ND

Quality Control: _____

Date: 04 MAY 95

Project Manager _____

Date: 5 May 95

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CERTIFICATE OF ANALYSIS
FINAL CONTAINER
STROMA FREE HEMOGLOBIN

LOT #: 950111-00-S

ASSAY	RESULT
Total Hemoglobin	13.7 g/dl
Met Hemoglobin	0.59 %
P_{50}	11.25 Torr
pH	7.42
Osmolarity	49 mOsm
Free Iron	1.22^{-5} mol Fe/ mol Heme
Phospholipid	<0.1 µg/ml
LAL	<0.03 EU/ml
Sterility	PASS

Quality Control: _____
Date: 17 JULY 95

Project Manager: _____ PhD SBB(ASCP)
Date: 17 July 1995

B411-201-04 (2/27/95)

BIONETICS

ORIGINAL

COPY

CERTIFICATE OF ANALYSIS
FINAL CONTAINER
α-α CROSS LINKED HEMOGLOBIN

LOT #: 950207-00-X

ASSAY	RESULT
Total Hemoglobin	6.78 g/dl
Met Hemoglobin	1.40 %
P_{50}	29.0 Torr
FPLC	>95 %
HPLC	>95 %
pH	7.86
Osmolarity	290 mOsm
Free Iron	2.17×10^{-5} mole Fe/ mole Heme
Phospholipid	<0.1 µg/ml
LAL	0.125 EU/ml
Sterility	PASS
Pyrogen	PASS

Quality Control: _____
Date: 17 July 95

Project Manager: _____, PhD, BCP (ASCP)
Date: 18 July 1995

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CERTIFICATE OF ANALYSIS
FINAL CONTAINER
α-α CROSS LINKED HEMOGLOBIN

LOT #: 950314-00-X

ASSAY	RESULT
Total Hemoglobin	8.24 g/dl
Met Hemoglobin	2.36 %
P_{50}	29.5 Torr
FPLC	>95 %
HPLC	>95 %
pH	7.52
Osmolarity	282 mOsm
Free Iron	3.01×10^{-5} mole Fe/ mole Heme
Phospholipid	1.0 µg/ml
LAL	0.25 EU/ml
Sterility	PASS
Pyrogen	PASS

Quality Control: _____
Date: 17 JUL 95

Project Manager: _____, PhD, SBB(ASCP)
Date: 18 July 1995

B411-201-04 (2/27/95)

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CERTIFICATE OF ANALYSIS
FINAL CONTAINER
α-α CROSS LINKED HEMOGLOBIN

LOT #: 950404-00-X

ASSAY	RESULT
Total Hemoglobin	7.48 g/dl
Met Hemoglobin	2.88 %
P_{50}	24.62 Torr
FPLC	87 %
HPLC	98.45 %
pH	6.90
Osmolarity	289 mOsm
Free Iron	3.55×10^{-5} mole Fe/ mole Heme
Phospholipid	0.7 µg/ml
LAL	0.125 EU/ml
Sterility	PASS
Pyrogen	PASS

Quality Control: _____
Date: 19 JUL 95

Project Manager: _____, PhD, SBB (ASCP)
Date: 19 July 1995

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CERTIFICATE OF ANALYSIS
FINAL CONTAINER
STROMA FREE HEMOGLOBIN

LOT #: 950516-01-S

ASSAY	RESULT
Total Hemoglobin	14.0 g/dl
Met Hemoglobin	0.64 %
P_{50}	11.5 Torr
pH	7.35
Osmolarity	66 mOsm
Free Iron	1.75^{-5} mol Fe/ mol Heme
Phospholipid	0.54 µg/ml
LAL	0.03 EU/ml
Sterility	PASS

Quality Control: _____
Date: 12/8/94 95

Project Manager: _____ PhD SBB(ASCP)
Date: 11 July 1995

ORIGINAL COPY

CERTIFICATE OF ANALYSIS
FINAL CONTAINER
α-α CROSS LINKED HEMOGLOBIN

LOT #: 950516-02-X

ASSAY	RESULT
Total Hemoglobin	12.30 g/dl
Met Hemoglobin	4.63 %
P_{50}	29.4 Torr
FPLC	>95%
HPLC	>95 %
pH	7.28
Osmolarity	278 mOsm
Free Iron	1.67×10^{-5} mole Fe/ mole Heme
Phospholipid	0.45 µg/ml
LAL	0.25 EU/ml
Sterility	PASS
Pyrogen	PASS

Quality Control: _____
Date: 17 July 95

Project Manager: _____
Date: 17 July 1995

B411-201-04 (2/27/95)

BIONETICS

ORIGINAL COPY

CERTIFICATE OF ANALYSIS
FINAL CONTAINER
α-α CROSS LINKED HEMOGLOBIN

LOT #: 950725-00-X

ASSAY	RESULT
Total Hemoglobin	14.96 g/dl
Met Hemoglobin	4.74 %
P_{50}	21.5 Torr
FPLC	>95%
HPLC	>80 %
pH	7.37
Osmolarity	275 mOsm
Free Iron	1.93×10^{-6} mole Fe/ mole Heme
Phospholipid	1.0 µg/ml
LAL	0.125 EU/ml
Sterility	PASS
Pyrogen	PASS

Project Manager: L B Lyttle PhD, SBB(ASCP)

Date: 29 August 1995

ORIGINAL COPY

CERTIFICATE OF ANALYSIS
FINAL CONTAINER
STROMA FREE HEMOGLOBIN

LOT #: 950808-00-S

ASSAY	RESULT
Total Hemoglobin	15.80 g/dl
Met Hemoglobin	0.58 %
P_{50}	5.25 Torr
pH	7.25
Osmolarity	263 mOsm
Free Iron	4.39×10^{-5} mole Fe/mole Heme
Phospholipid	<0.1 µg/ml
LAL	0.25 EU/ml
Sterility	PASS

Project Manager: _____

Date: 15 Sep 95

HEMOGLOBIN INVENTORY

TYPE	LOT #	VOLUME (liters)	FORMULATION
Ao			
	US94HP01	10	HemAzero(Hemosol)
Stroma Free	94242	4.2	
→	950111-00-S	24.008	Phosphate
→ NRL	950516-01-S	0	Phosphate
→ NRL	950808-00-S	19.825	Saline
Cross Linked	91007	2	RA
	91119	6.6	RA
	91274	500 ml	RA
	92057	4.8	RA
	92134	300 ml	Phosphate
	92141	2.6	CnMet
→	J94319	10.5	RA
→	950207-00-X	16.5	RA
→	950314-00-X	26.7	RA
→	950404-00-X	21.108	RA
→ NRL	950516-02-X	0	Saline
→ NRL	950725-00-X	5.3	Saline

→ Inventory manufactured under contract DAMD17-94-C-4154.

2 October 95

BLOOD SOURCE REPORT

PERIOD: FISCAL YEAR 1995

SOURCE	QTR 1	QTR 2	QTR 3	QTR 4	CUM
WRAMC		62	54	46	162
FAMC		91	151	161	403
MAMC		22	19	18	59
CAMP MEMORIAL / KNOX	60			48	108
BLOOD BANK CENTER / HOOD		30			30
NOBLE ACH		21	78	17	116
ASWBPL / MCQUIRE	130	61	18	15	224
NATIONAL NAVAL MEDICAL CENTER		113	37	62	212
PORTSMOUTH NAVAL MED CEN				10	
WILFORD HALL AIR FORCE MED CEN				11	
NIH CLINICAL CENTER				31	
TOTAL	190	400	357	419	1366